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DNA Adducts in Peripheral Blood Lymphocytes from Aluminum Production Plant Workers Determined by ^{32}P-Postlabeling and Enzyme-Linked Immunosorbent Assay

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^{32}P-Postlabeling analysis and enzyme-linked immunosorbent assay (ELISA) have been used to detect DNA adducts in peripheral blood lymphocytes from primary aluminum production plant workers who were exposed occupationally to a mixture of polycyclic aromatic hydrocarbons (PAHs). Preliminary results reported here are from a comparative study being performed in two aluminum plants. The levels of aromatic DNA adducts have been determined by the ^{32}P-postlabeling assay in samples collected on two occasions, 1 year apart. PAH–DNA adduct levels have also been determined by competitive ELISA in the second set of DNA samples. The results show the necessity of follow-up biomonitoring studies to detect possible alterations in biological effect induced by changing exposures. The comparison of the results obtained by ^{32}P-postlabeling and ELISA may lead to a better understanding of the power and weaknesses of the two methods applied in these studies.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants that are strongly suspected of exhibiting carcinogenic effects in occupationally exposed populations such as coke oven and iron foundry workers, roofers, and primary aluminum plant workers (*1,2*). Many of these compounds have been shown to be metabolized to reactive derivatives that bind covalently to cellular macromolecules. Because formation of carcinogen–DNA adducts is considered to be a necessary, early step in tumor initiation, monitoring levels of DNA adducts may contribute to a more reliable risk assessment than determination of the exposure dose only (*3*).

Among the numerous recent studies on populations occupationally exposed to PAHs, DNA adduct levels in peripheral blood lymphocytes from aluminum plant workers have been investigated only by synchronous fluorescence spectrophotometry (*4*), and very recently in our laboratories by ^{32}P-postlabeling (*5*). This latter study (study 1) has been expanded to include a second and larger set of blood samples from workers from the same aluminum plants and both the ^{32}P-postlabeling assay and competitive ELISA have been used for the analysis of the DNA samples (study 2). Preliminary results and evaluation of the comparative study are presented here.

Materials and Methods

In study 1, blood samples were obtained from 46 male workers at two Hungarian primary aluminum production plants and from 29 occupationally unexposed individuals. In study 2, 172 workers in the same two aluminum plants were monitored 1 year later and the results compared with adduct analyses of 127 samples from a blood bank presumably from occupationally unexposed individuals. Subjects from the aluminum plants represented various job categories. Mean age and length of employment of the workers and age of the controls were similar in each group (data not shown).

Peripheral blood lymphocytes were isolated from 20 to 40 mL of blood by gradient centrifugation on Ficoll 400-Uromiro within a few hours after blood was drawn, and DNA was isolated as described previously (*5*). Samples of DNA were analyzed for aromatic DNA adducts by ^{32}P-postlabeling using nuclease P1 digestion to enhance sensitivity (*5,6*). PAH–DNA adducts were determined by competitive ELISA with fluorescent end point as described previously (*7*) using a rabbit antiserum elicited against DNA modified with benzo[*a*]pyrene (*8*).

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Statistical analysis of the data was carried out with Mann-Whitney U tests for the ^{32}P-postlabeling studies and chi-square tests for the ELISA study. Results obtained by ^{32}P-postlabeling and ELISA in the same set of DNA samples were compared using the rank correlation test.

Results

^{32}P-Postlabeling

Autoradiographic patterns of many of the DNA samples showed characteristic diagonal arrangements of partly resolved or well-resolved spots or a diffuse band of the ^{32}P-labeled adducted nucleoside 3',5'-bisphosphates, as shown in Figure 1.

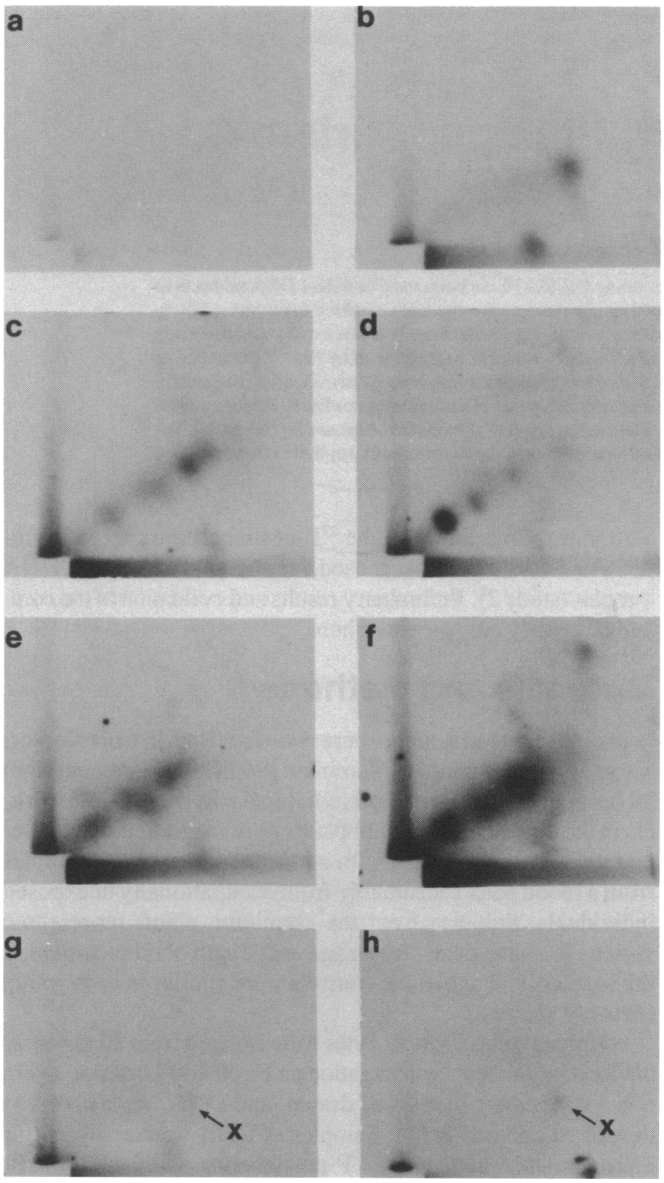

FIGURE 1. Characteristic autoradiographic maps of ^{32}P-labeled digests of DNA from peripheral blood lymphocytes from aluminum production plant workers (*a–f*) and unexposed controls (*g* and *h*). Chromatography was on PEI-cellulose TLC sheets and autoradiography was at −70°C for 4 days.

There was a remarkable similarity in the most frequent types of patterns and in the relative mobility of some well-resolved main spots in studies 1 and 2.

Aromatic DNA adduct levels in the occupationally exposed populations varied in a range of up to 7.1 adducts/10^8 nucleotides (study 1) and 9.6 adducts/10^8 nucleotides (study 2), with distributions significantly different from normal ($p < 0.05$). The adduct detection limit was approximately 0.5 adducts/10^8 nucleotides. For the samples with no detectable adducts, nominal adduct levels were calculated from the radioactivities in an average-size diagonal area of the TLC sheets in the region of adduct migration.

In study 1 there was no significant difference between the mean DNA adduct levels of the control group and of the individuals in plant 1. However, the mean DNA adduct level obtained from the blood samples of the workers in plant 2 was significantly higher ($p < 0.001$) than both that of the controls and that of plant 1 (Table 1) (*5*). One year later, in study 2, results indicated a significant elevation ($p < 0.001$) of the mean DNA adduct level from workers in plant 1 as compared to the level in the first study. However, the mean DNA adduct level for workers in plant 2 was unchanged (Table 1). This finding was confirmed by a comparison of 2 subgroups of workers who participated in both studies 1 and 2. The groups included 9 and 19 individuals from plants 1 and 2, respectively. The plant 1 workers exhibited a significant increase ($p < 0.001$) in mean levels of DNA adducts from one year to the next, whereas no significant change occurred ($p = 0.17$) in the plant 2 subgroup (Table 1). Slightly elevated DNA adduct levels were observed in smokers from plant 1 in study 1 ($p < 0.05$) (*5*) but not in workers from the same plant in study 2. Plant 2 could not be similarly evaluated because of the low number of nonsmoking individuals in that workplace.

ELISA

Study 2 comprised 104 samples from plant 1, 23 samples from plant 2, and 127 samples from a blood bank. PAH–DNA adducts were also determined by competitive ELISA from the same DNA

Table 1. Levels of aromatic DNA adducts determined by ^{32}P-postlabeling and ELISA in peripheral blood lymphocytes from aluminum production plant workers.

	DNA adducts/10^8 nucleotides (mean ± SD)		
	Study 1	Study 2	
	^{32}P-Postlabeling	^{32}P-Postlabeling	ELISA
Aluminum plant 1			
Range	0.3 - 4.1	0.5 - 9.2	4.0 - 30.0
All donors	1.5 ± 1.0 (25)[a]	3.1 ± 1.8 (127)	8.0 ± 5.2 (104)
Subgroup	1.3 ± 0.9 (19)	4.1 ± 1.9 (19)	
Aluminum plant 2			
Range	0.4 -7.1	0.9 - 9.6	4.0 - 14.6
All donors	3.1 ± 1.7 (21)	3.0 ± 1.7 (45)	6.4 ± 4.0 (23)
Subgroup	3.4 ± 1.6 (9)	2.7 ± 1.2 (9)	
Controls			
Range	0.2 - 2.4	ND	4.0 - 25.3
All donors	1.3 ± 0.5 (29)		5.1 ± 5.0 (127)

ND, not determined (analysis in progress).
[a]Number of individuals in parentheses.

Table 2. PAH-DNA adducts determined by ELISA in peripheral blood lymphocytes from aluminum production plant workers.

Group	Total	Negative[a]	Positive
Plant 1	104[b]	29 (28%)	75 (72%)
Plant 2	23	9 (39%)	14 (61%)
Controls	127	80 (63%)	47 (37%)

[a]Fewer than 4.0 adducts/10^8 nucleotides.
[b]Number of individuals.

samples used for ^{32}P-postlabeling analysis. The detection limit was approximately 0.12 fmole adduct/μg DNA or 4.0 adducts/10^8 nucleotides in the majority of the assays. Therefore, samples below this value were considered negative. Whereas 37% of the control DNA samples were over the detection limit, 72% and 61% of the samples from plant 1 and plant 2, respectively, were positive (Table 2). These differences proved to be significant in comparison to the control group ($p < 0.001$ and $p < 0.05$, respectively), but there was essentially no difference between workers in the two aluminum plants ($p > 0.1$). DNA adduct levels ranged as high as 0.9 fmole adduct/μg DNA or 30 adducts/10^8 nucleotides. Mean DNA adduct levels are shown in Table 1. Levels of DNA adducts in positive samples demonstrated no difference between smokers and nonsmokers. Values were 9.7 ± 3.7 adducts/10^8 nucleotides for smokers and $11.5 \pm 5.3/10^8$ for nonsmokers in plant 1, and $9.3 \pm 3.9/10^8$ for smokers and $10.9 \pm 5.4/10^8$ for nonsmokers in the control group.

Discussion

The two techniques that have been applied here for the detection of carcinogen-DNA adducts are ^{32}P-postlabeling, which has a broad specificity for aromatic DNA adducts (*9*), and ELISA, which has been shown to detect DNA adducts of a variety of PAHs (*10*).

The ^{32}P-postlabeling data demonstrate that the assay is sensitive enough for biomonitoring occupational PAH exposure in the primary aluminum industry. In study 1 the significant difference between the two aluminum plants was primarily attributed to known differences in the design of the technology and higher levels of PAHs in the older plant (plant 2) (*5*). In the follow-up study 1 year later, a significant elevation of the DNA adduct levels was revealed in the more modern plant, whereas there was no apparent change in the older plant. This finding was strengthened by the comparison of subgroups of workers from each plant who participated in both studies. Factors other than the specific plant technology that may have influenced these results include source and composition of anode materials and qualitative alteration of the mixture of airborne PAHs. Analysis of PAH-DNA adducts by ELISA and ^{32}P-postlabeling have resulted in the same major conclusions. Significant differences were demonstrated between unexposed and exposed groups, and the two exposed groups were found to be similar to each other. In addition, neither ^{32}P-postlabeling, nor ELISA demonstrated in smokers and nonsmokers detectable differences in adduct levels.

Although there was a remarkable agreement between the two DNA adduct assays in the recognition of relative differences on a group basis, a diversity was found in the corresponding numerical values on an individual basis. The overall detection limit was approximately 8-fold lower by the ^{32}P-postlabeling assay, and the maximum values were about 3-fold higher by the antibody assay. In spite of the much broader adduct specificity of the ^{32}P-postlabeling technique, mean adduct values were 2- to 3-fold higher by the immunoassay. By using rank correlation test, statistical evaluation of the positive samples by ELISA and of their corresponding ^{32}P-postlabeled pairs indicated a weak but significant correlation between the two methods ($r_{rank} = -0.219$; $p < 0.05$). The diversity may primarily come from different labeling efficiencies of aromatic DNA adducts by ^{32}P-postlabeling and differential recognition of PAH-DNA adducts by ELISA. Therefore, although there is inevitably a degree of uncertainty surrounding absolute quantitation by either method, each method is internally consistent, and statistically significant differences in adduct levels between exposed groups and controls were clearly demonstrated both by ^{32}P-postlabeling and immunoassay.

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